General Purpose Segmentation for Microorganisms in Microscopy Images

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General Purpose Segmentation for Microorganisms in Microscopy Images

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Abstract: In this paper, we propose an approach for achieving generalized segmentation of microorganisms in microscopy images. It employs a pixel-wise classification strategy based on local features. Multilayer perceptrons are utilized for classification of the local features and is trained for each specific segmentation problem using supervised learning. This approach was tested on five different segmentation problems in bright field, differential interference contrast, fluorescence and laser confocal scanning microscopy. In all instance good results were achieved with the segmentation quality scoring a Dice coefficient of 0.831 or higher.

1 INTRODUCTION

Microscopy is the art of observing objects which are normally too small to be seen by the unaided human eye. It is one of the most important information gathering tools in many different fields such as microbiology and have remained so since it's conception over a hundred years ago. One of the main applications of microscopy is the observation of microorganisms, an important endeavor in microbiology and medical science. Through visual magnification of a 100 times or more, a wealth of visual information can be extracted from even the tiniest specimens or samples. In fact information can be so plentiful that a thorough analysis can be quite a time consuming task. Combining microscopy with additional techniques such as time-lapse videos and z-layering, can make the amount of information even more staggering. This calls for means by which the analysis tasks may be partially or fully automated. Fortunately many microscopy analysis tasks can, on their base level, be boiled down to locating instances of one or several specific classes within an image, examples of this includes cancer (Wienert et al., 2012) and malaria diagnostics (F. Boray Tek and Kale, 2009a). This means that the bulk of the analysis work can be automated by developing a general method for detection microorganisms within microscopy images. Unfortunately this can be a quite challenging problem due to visual variation, which stems from the employed microscopy type and specific species of microorganism. A few examples of this behavior can be observed in figure 1.

In this positional paper we will present the preliminary work for a single segmentation method that is capable of handling many of these visually varying problems.

2 Related Work

Automatic microscopy image analysis is by no means a new topic of research and material on the matter have been published for more than 50 years. Regarding the specific subject of cell segmentation, a survey is illustrated in figure 2 which nicely details the number article published on cell segmentation every 5 years as well as their overall methodology for solving the problem. Now it clearly shows that the amount of research gone into cell segmentation has been steadily increasing over the last 50 years, indicating a clear interest in solving the problem. However the lower part of figure 2 illustrates a major issue with the research that has been performed; it suffers from a very divergent methodology. Amongst

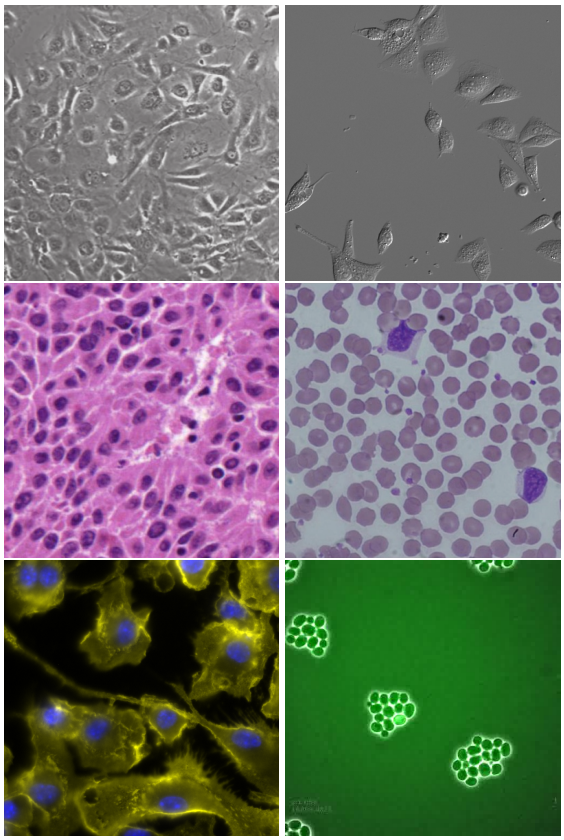

Figure 1: Examples of the visual variety posed by different species of microorganisms and types of microscopy. Contains images produced using bright field(Wienert et al., 2012)(bin Abdul Jamil et al., 2012), differential interference contrast, fluorescence(Institute, 2013) and phase contrast microscopy(Kane et al., 2013).

the utilized methods for achieving microorganism detection are variations of watershed segmentation (Lebrun et al., 2007) (Ao et al., 2011) (Cheng and Rajapakse, 2009) (bin Abdul Jamil et al., 2012), contour and shape based segmentation (Wienert et al., 2012) (Zhou, 2007) (Kujiper and Heise, 2008), color and intensity based segmentation (Zhaozhen Ying and Kanade, 2010) (F. Boray Tek and Kale, 2009b) (F. Boray Tek and Kale, 2009a), wavelet based detection (Ariel J. Bernal and Bernal, 2008) and shape based detection (Kevin Smith and Lepetit, 2009). Additionally there is a clear trend towards specialized solutions, as stated in (Meijering, 2012) and which may be observed in the previously mentioned work. The program CellProfiler is capable of handling many microorganism detection problems, however it does so by simply letting the user manually choose the specific segmentation method which is to be employed(Carpenter et al., 2006).

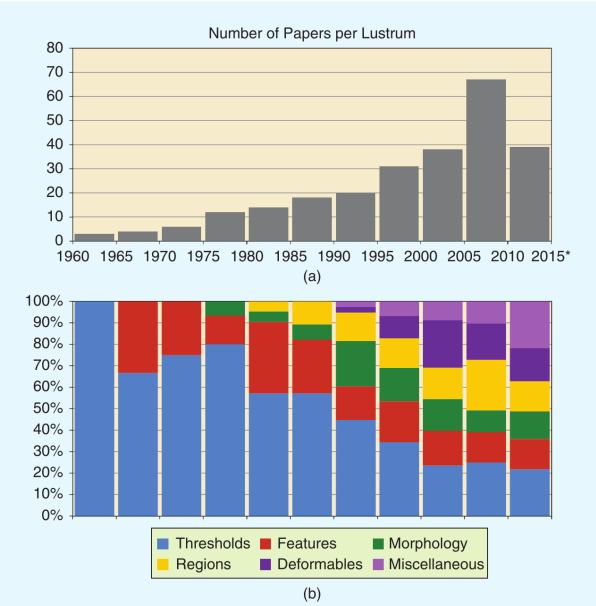

Figure 2: This figure illustrates the progression of research in cell segmentation over the past 50 years, both in terms of quantity and methodological distribution. It shows that no real consensus has been achieved on the approach for cell segmentation. The graph was obtained from (Meijering, 2012).

This article will contribute to the overall state of the art by introducing a single segmentation method that may determine an optimal segmentation rule for a specific microorganism detection problem. Segmentation is chosen as the base of the detection strategy as it is capable of dealing with the great variations in shape, orientation and appearance that microorganism naturally exhibit in microscopy images.

3 Method

The main principle of this work is the collection and classification of pixel-wise local features. This is accomplished by having a machine learning algorithm determine the optimal classification rule through supervised learning. The reason for this approach is that it provides a simple framework with a constant structure capable of handling a wide array of microorganism segmentation problems, as long as the objects are locally distinguishable.

3.1 Features

It is difficult to ensure that a given feature set can distinguish between background and object in all plausi-

ble situation. However, it was experimentally found that in many specific problems good segmentation could be achieved using a feature set based on color and gradient information. It is defined below,

$$\bar{F}(x,y) = \begin{pmatrix} \bar{I}(x,y) \\ \mathrm{E}[\bar{I}(x,y)] \\ \mathrm{Var}[\bar{I}(x,y)] \\ \bar{g}(x,y) \\ \mathrm{E}[\bar{g}(x,y)] \\ \mathrm{Var}[\bar{g}(x,y)] \end{pmatrix} \quad (1)$$

Where,

$\bar{F}(x,y)$ is the pixel feature-vector at (x,y),
$\bar{I}(x,y)$ is the pixel value at (x,y),
$\bar{g}(x,y)$ is the pixel gradient magnitude at (x,y),

Both the pixel value and gradient magnitude functions are denoted as being vectors as pixel values are assumed to be defined in the HSV color space. The expectation and variance operators both refer to element wise operations. The feature is evaluated on a square area centered on the pixel in question. Evaluating both variance and mean can get quite computationally expensive for larger areas. To remedy this problem a moving average technique is utilized to drastically improve real-time performance. For example the mean may be efficiently evaluated using the following equation,

$$\begin{aligned} \bar{\mu}(x+1,y) =& \bar{\mu}(x,y) \\ &+ \frac{1}{(2w+1)^2}\left[\sum_{j=-w}^{w} \bar{I}(x+w+1,y+j)\right. \\ &\left. - \sum_{j=-w}^{w} \bar{I}(x-w,y+j)\right] \end{aligned} \quad (2)$$

Where,

$\bar{\mu}_I(x,y)$ is the mean value of pixel (x,y),
$\bar{I}(x,y)$ is the value of pixel (x,y),
w is the width of the square evalutation area

3.2 Classification

Multilayer perceptrons with sigmoid activation functions was utilized for pixel classification in this method, which contained a single input, output and hidden layer. Each neuron within the network is defined as,

$$a_j = f\left(c_j \sum_i w_{i,j} a_i\right) \quad (3)$$

Where,

a_j is the output of the jth neuron,
f is an activation function,
c_j is the neuron gain,
a_i is the ith input to the neuron,
$w_{i,j}$ is the weight of the edge from ith to jth neuron.

As this network is used for classification, the activation function for each neuron within the hidden and output layer is a sigmoid function which is defined below,

$$f(z) = \frac{1}{1+e^{-z}} \quad (4)$$

In this particular work the multilayer perceptrons is used to estimate the likelihood of a given feature belonging to either the background or object class. This means that the network has two output nodes for each class, each of which outputs the likelihood for one of the given classes. In the training phase, the output corresponding to a given training sample feature is either [0, 1] or [1, 0] depending on the class which the feature belongs to. The final classification is then performed using Bayesian decision,

$$\mathrm{class}(\bar{x}) = \begin{cases} O & \text{if } P(\mathrm{O})P(\bar{x}|\mathrm{O}) > P(\mathrm{B})P(\bar{x}|\mathrm{B}) \\ B & \text{else} \end{cases} \quad (5)$$

Where,

$\bar{x}$ is an input feature,
O is the object class,
B is the background class,

A binary image is achieved by numerically representing the background and object class respectively as 0 and 1. The network is trained using a variation of the standard backpropagation, whose main difference lies on that it uses both first and second order derivatives to estimate the optimal search direction during optimization of neuron weight. At iteration n the optimal search direction is,

$$\bar{p}_n = -\mathbf{H}_n^{-1} \bar{g}_{w,n}. \quad (6)$$

Where,

$\bar{p}_n$ is the optimal search direction,
$\mathbf{H}_n$ is the Hessian matrix,
$\bar{g}_{w,n}$ is the gradient.

Now this search direction estimate leads to fewer iteration before convergence, but both the Hessian and it's inverse are relative expensive to estimate(Nawi et al., 2006). To remedy this problem the Broyden-Fletcher-Goldfarb-Shanno (BFGS) recursive iteration

scheme is used to accurately approximate the Hessian using the following set of equations(Nawi et al., 2006),

$$\bar{s}_n = \bar{w}_{n+1} - \bar{w}_n \tag{7}$$

$$\bar{y}_n = \bar{g}_{w,n+1} - \bar{g}_{w,n} \tag{8}$$

$$\mathbf{H}_{n+1}^{-1} = \mathbf{H}_n^{-1} + \left(1 + \frac{\bar{y}_n^T \mathbf{H}_n^{-1} \bar{y}_n}{\bar{s}_n^T \bar{y}_n}\right) \frac{\bar{s}_n \bar{s}_n^T}{\bar{s}_n^T \bar{y}_n} - \frac{\bar{s}_n \bar{y}_n^T \mathbf{H}_n^{-1}}{\bar{s}_n^T \bar{y}_n} \tag{9}$$

Where,

$\bar{w}_n$ is a vector containing the neuron weights.

As long as $\mathbf{H}_n^{-1}$ is initialized as a positive definite matrix, the above will converge towards the true Hessian(Nawi et al., 2006). The training algorithm is described in detail in (Nawi et al., 2006). It is important that the training algorithm is supplied with an equal amount of samples from each class. Having one class vastly overrepresented leads to classification bias, an effect which is studied in detail in (Brain, 2003). We therefore subsample the overrepresented class.

4 Experimental Results

The method has been tested on four image sets containing different microorganisms and produced using different microscopy types. Each image set contains approximately 20 images and the results have been achieved by running the segmentation method on all of them and performing comparisons to expert produced ground truths. The main purpose is to illustrate the viability of the method on a variety of different detection problems. Segmentation quality is gauged using the Dice coefficient rather than pixel-wise classification error, it is defined below (Ao et al., 2011),

$$D = \frac{2 \cdot |S_{\text{auto}} \bigcap S_{\text{manual}}|}{|S_{\text{auto}} + S_{\text{manual}}|} \tag{10}$$

Where,

D is the Dice coeffecient,

S_{auto} is the set of segmentation object pixels,

S_{manual} is the set of ground truth object pixels.

Image Set	Dice Subsample
Fluorescence	0.831
Bright Field	0.851
Laser Scanning	0.952
DIC Wound	0.963

Table 1: Table of experimental test results.

Now the dice coefficient for each image set was estimated by utilizing a k-fold procedure with 4 folds. A example image along with an example segmentation for each image set can be seen in figure 3, 4, 5 and 6. The image sets were obtained from online microscopy image databases provided by (Institute, 2013) and (for Bio-Image Informatics, 2013). The results of each test can be observed in table 1. In all test high Dice coefficients were achieved, this indicates that the method is very versatile and can handle a wide range of segmentation problems. The results obtained from the DIC Wound image set can be directly compared to those achieved by (Zaritsky et al., 2011), who designed a segmentation algorithm for this particular problem. In their work they achieved an average pixel-wise segmentation accuracy of 0.922, which is very much comparable to the Dice coefficient of 0.963 obtained by our method. In (Ao et al., 2011) a method is presented for segmenting cancer cells in microscopy images. They achieved an average Dice coefficient of 0.9 which was deemed to be an acceptable degree of accuracy. While the data set used in (Ao et al., 2011) was not available for testing our method thus preventing a direct comparison, it does indicate the needed level of precision. As the results obtained with our method are very close to the results obtained in (Ao et al., 2011), this demonstrates that our method is capable of producing segmentation quality on par with others, without being specifically designed for a particular problem.

5 Conclusion

In this paper it has been shown that a wide range of segmentation problems in microscopy can be solved through pixel-wise classification of local features. These are based on the mean and variance of color and gradient magnitude evaluated in an area centered locally on each pixel. A classifier, in this case multilayer perceptrons, is automatically trained to each specific segmentation problem, using user labeled sample features. In order to document the viability of the method, it was tested on five different image set produced using bright field, fluorescence, differential interference contrast and laser confocal scanning microscopy. In all cases good results were obtained with a Dice coefficient ranging from 0.831 to 0.963.

In future work this method will be subjected to more systematic tests in order to closely investigate it's properties. Additionally more data produced using different kinds of microscopy and microor-

Figure 3: Example segmentation of an image from the Bright Field set. Input image at the top, ground truth in the middle and segmentation at the bottom.

ganisms will be utilized in order to fully gauge the potential and limits of the method.

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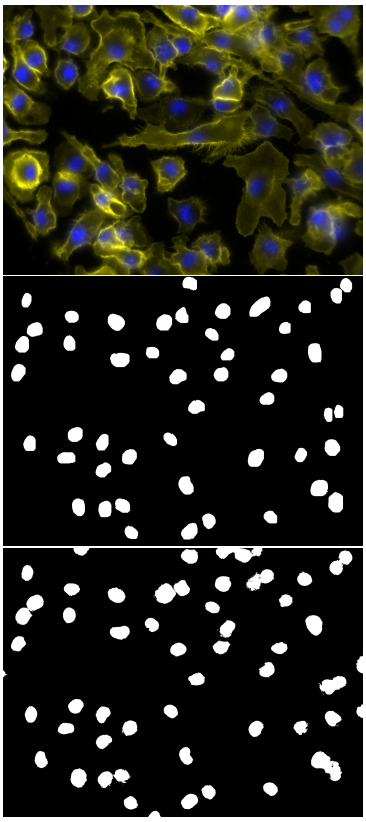

Figure 4: Example segmentation of an image from the Fluorescence set. Input image at the top, ground truth in the middle and segmentation at the bottom.

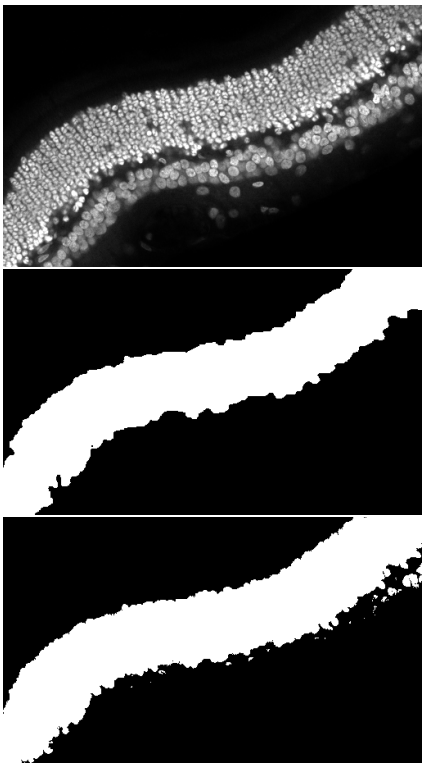

Figure 5: Example segmentation of an image from the Laser Scanning set. Input image at the top, ground truth in the middle and segmentation at the bottom.

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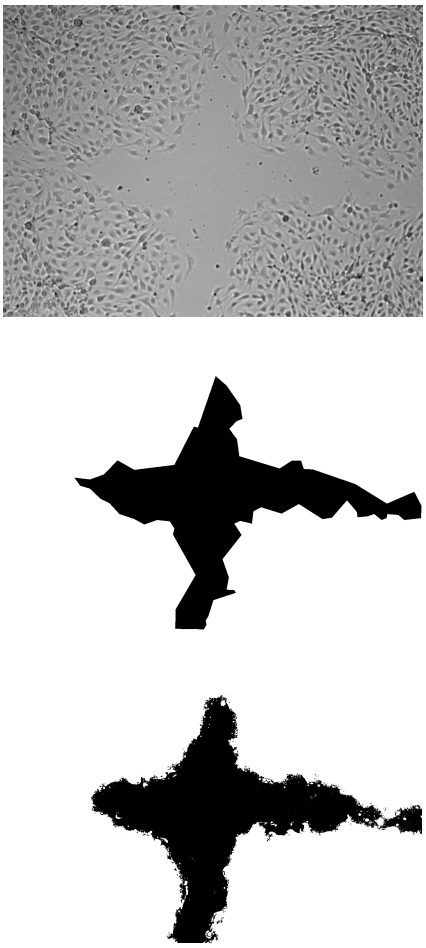

Figure 6: Example segmentation of an image from the DIC Wound set. Input image at the top, ground truth in the middle and segmentation at the bottom.

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